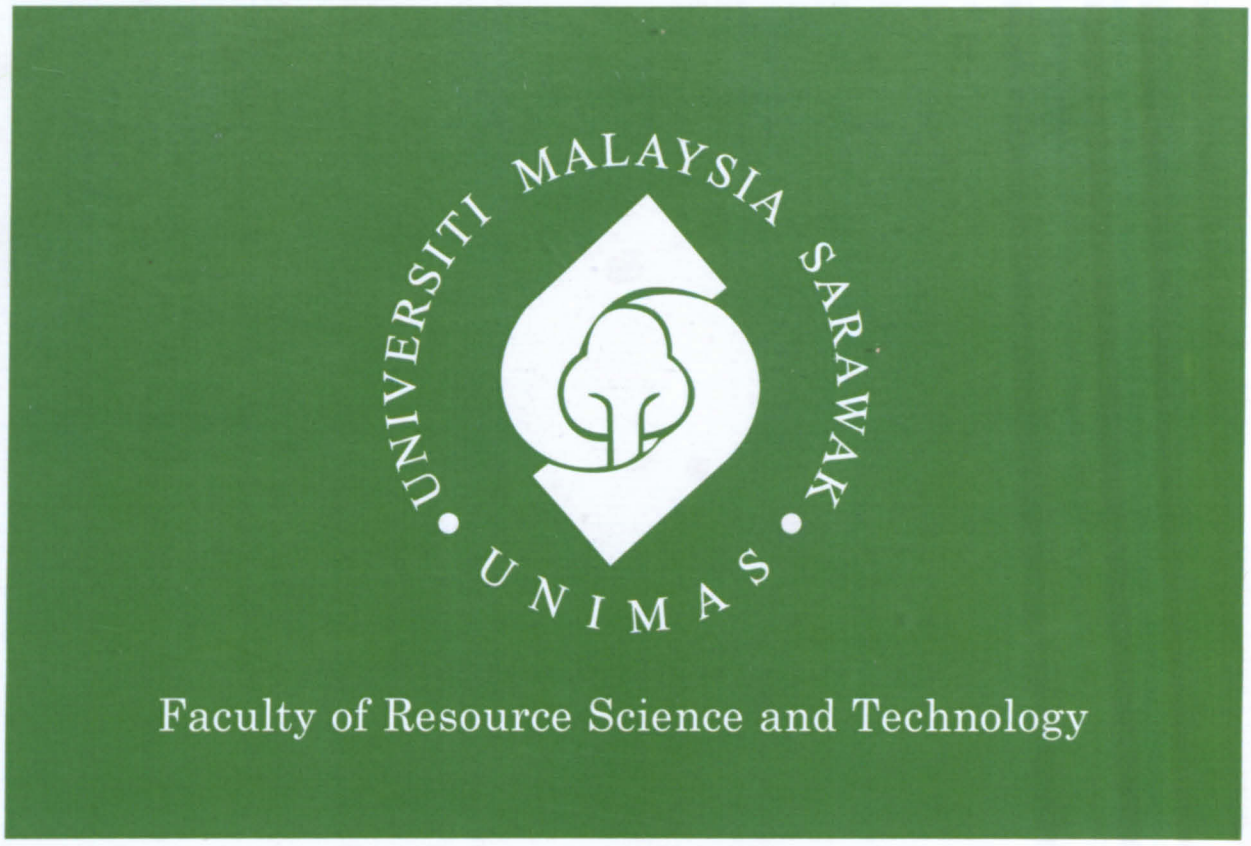

Quantifying Shrinkage of Morphometric Measurements: Museum Specimen Versus Livetrapping Specimen of Bulbul (Family: Pycnonotidae)

Floyd Florence
(30235)

Bachelor of Science with Honours
(Animal Resource Science and Management)
2015

UNIVERSITI MALAYSIA SARAWAK

Grade: _____

Please tick (√)

Final Year Project Report ☑

Masters ☐

PhD ☐

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This declaration is made on the 10 day of July 2012.

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Quantifying Shrinkage of Morphometric Measurements: Museum Specimen Versus Livetrapping Specimen of Bulbul (Family: Pycnonotidae)

FLOYD FLORENCE

(30235)

This project is submitted in fulfillment of the requirements for the Degree of Bachelor of Science with Honours (Animal Resource Science and Management)

DEPARTMENT OF ZOOLOGY

FACULTY OF RESOURCE SCIENCE AND TECHNOLOGY

UNIVERSITI MALAYSIA SARAWAK

2015

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No portion of the work referred to in this dissertation has been submitted in support of an application for another degree of qualification of this or any other university of institution of higher learning.

Floyd Florence

Your Full Name

Animal Resource Science and Management

Faculty of Resource Science and Technology

Universiti Malaysia Sarawak

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Quantifying Shrinkage of Morphometric Measurement: Museum Specimen Versus Livetrapping Specimen of Bulbul (Family : Pycnonotidae)

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Animal Resource Science and Management Programme

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ABSTRACT

Bulbul (Family: Pycnonotidae) are monomorphic birds. Frequently morphometric study was conducted by using museum dry specimen of bulbuls. As body measurement of museum specimen tend to shrink, it is unwise to ignore it. This study was design to compare whether there are significant different in shrinkage between live and museum specimen. Seven external morphological parameters of bulbul were measured. Live and museum number of sample was equaled using Random Number Generator from Microsoft Excel and all the body measurement were analysed using SPSS software using independent t-test and Mann-Whitney U test. The significant difference were found in bill width and head bill of olive-winged bulbul (*P.plumosus*), bill length of puff-backed bulbul (*P.eutilotus*) and bill length, bill width, bill depth and head bill of yellow-vented bulbul (*P. goiavier gourdini*). As for the amount of shrinkage, it were found ranging from 0.35% to 15.48% for olive-winged bulbul, -14.32% to 10.04% for puff-backed bulbul and 1.52% to 37.80% for yellow-vented bulbul.

Keywords: Pycnonotidae, monomorphic, shrinkage, morphological, t-test, Mann-Whitney U test.

Bulbul (Famili: Pycnonotidae) adalah monomorfik. Selalunya kajian morphometric telah dijalankan dengan menggunakan muzium spesimen kering bulbuls. Seperti yang kita ketahui, ukuran badan spesimen muzium akan mengecut dan ia adalah tidak bijak untuk mengabaikannya. Kajian ini dibuat untuk membandingkan sama ada terdapat perbezaan yang signifikan di antara pengecutan spesimen hidup dan muzium . Tujuh parameter morfologi burung "bulbul" telah diukur. Jumlah Spesimen hidup dan spesimen musium telah disamakan menggunakan Random Number Generator dari Microsoft Excel dan Semua data statistik telah dianalisa menggunakan perisian SPSS, menggunakan ujian-t tidak bergantung dan ujian Man-Whitney U. Perbezaan signifikan telah didapati pada ketebalan paruh dan kepanjangan kepala ke paruh untuk "olive-winged bulbul" (P. plumosus), kepanjang paruh untuk spesies "puff-backed bulbul" (P. eutilotus) manakala ketebalan paruh, kelebaran paruh, kepanjangan paruh dan kepanjang kepala ke paruh untuk "yellow-vented bulbul" (P. goiavier gourdini). Bagi jumlah pengecutan, ia telah didapati berada antara 0.35% hingga 15.48% untuk "olive-winged bulbul", -14.32% hingga 10.04% untuk spesies "puff-backed bulbul" dan 1.52% hingga 37.80% untuk "yellow-vented bulbul".

Kata kunci: 'Pycnonotidae', monomorfik, pengecutan, morfologikal, ujian-t, ujian Man-Whitney U

1.0 Introduction

Birds are most-known class of vertebrate animals which occur in almost all habitats worldwide. According to Symthies (1999), birds possessed one special characteristic which present of feathers. Smythies (1999) also recorded there are about 39 endemic species occur in Borneo. Birds are very fascinating creatures that play important part in ecosystem, as their behaviour changes provides clues to changes in the environment. In addition, birds play a significant role in both science and conservation purposes.

Bulbuls are belong to the family Pycnonotidae consists of 119 species (Gill, 1990). Most of bulbuls species distributed across Africa, Middle East, tropical Asia to Indonesia and north as far as Japan. They are frugivorous and also insect eaters (Mackinnon and Philips, 1993) which have short neck and pale coloured normally in yellow, orange, black or white pattern (Reduan *et al.*, 2003). Bulbuls are also one of the songster species which has very musical voices. Bulbul or any other bird that died during sampling, research or found dead will be preserved in the museum under a dry condition.

Frequently, museum specimen were widely used by many researcher to study the ecology, evolution, behaviour and taxonomy of birds (Wandeler *et al.*, 2007, Moktar, 2008, Ramji & Rahman, 2011). For example, One of important morphology for studying feeding ecology and subspecies classification is bill length (Hall, 1996). Thus, museum specimen will be used because the ease in assessing the sample and it is vital to have an accurate bill measurement. However, museum specimen are usually handled and preserved in different ways such as freezing, drying and skinning, which may cause some problem when being used for study (Kuczynski *et al.*, 2003). Specimen in the museum prone to shrink directly after the death and

this could lead to error in any studies if ignored. Some biologist recommended that there should be a standard measurement technique from museum specimen to be applied directly to living specimen. Mostly all previous study regarding bird morphometry have been using data from museum skins of the large museum (Cramps & Perrins, 1993). Thus, is is essential to test how representative such material is. Information regarding differences between external morphological characteristic of live and museum specimen on olive-winged bulbul, puff-backed bulbul and yellow-vented bulbul has not yet been address and investigate. Based on the current study thus far, no research on the difference between museum and live specimens measurement in Sarawak, causing the sexing modeling using measurements taken from museum specimen being applied blindly to live specimen. The study is important and vital as to give an overview on amount of shrinkage happen to external morphological characteristics of olive-winged bulbul, puff-backed bulbul and yellow-vented bulbul. Thus, further research could be done to make some correction factor to the museum measurements before proceed with sexing modeling.

1.1 Objectives

For this project, the main point to achieve are:

- To quantify the percentage amount of shrinkage to external morphological characteristics of Bulbul, based on museum and live specimen.
- To determine the significant differences of the morphological characteristics.
- To advice future morphometric sexing guideline, based on the amount of shrinkage of each respective morphometric characteristic.

1.2 Justification

Guidelines for analyzing the monochromatic species of birds through parameter measurements are frequently developed using specimen in museum. Despite the fact that these specimens tend to shrink when they dry, this shrinkage is typically ignored and the result of museum-based studies are directly applied to living birds. To determine whether shrinkage was affecting this parameter, is would have been necessary to compare live birds with museum specimen. The hypothesis of this studies is there are significant difference in the shrinkage level between live and museum bulbul specimen.

1.3 Hypothesis

Null hypothesis, H_o : There are no difference(s) in the external morphological measurement between live and museum specimen.

Alternative hypothesis, H_A : There are difference(s) in the external morphological measurement between live and museum specimen.

2.0 Literature review

2.1 Bulbuls (family: Pycnonotidae)

Bulbuls from family Pycnonotidae is medium sized passerine songbirds. They have a musical song and make a cup-shaped nest in tree (Mackinnon & Phillipps, 1993). According to Lekagul and Round (1991) bulbuls also noted as gregarious and can be found together in fruiting trees. Bulbul is a lower canopy bird except for Scaly-breasted or Ashy Bulbuls which is high canopy forms (McClure, 1988). Bulbuls are monomorphic birds (Reduan *et al.*, 2003) which are hard to distinguish between male and female.

Bulbul study of sexual dimorphism using external morphological characteristic was conducted be Reduan *et al.* (2003). Result showed that the male were greater than the female in bill morphology, tail length and wing length but for olive-winged bulbul no differences in selected external characters was found.

Another study made by Fairuz (2007), which used the museum specimen in Sarawak Museum to determine the gender of bulbul using measurement of external characteristic. Based on many precious study regarding about museum specimen, it tend to shrink and decrease in length which might cause error in measurement study. Thus, even the smallest decrease in length of certain body part can cause mistake in determining sex for monomorphic bird.

2.2 Shrinkage of Museum Specimen

Research performed in museum collections is very attractive because material is easily available but many problem may arise. One of the most discussed problems is skin shrinkage after preparation. Given that shrinkage level could reach to an average of as much as 4% of living length of various body components (Fjeldsa, 1980), ignoring this case could be unwise. Therefore, appearance in shrinkage should be expected in any study based on sample size of old specimen. The degree of shrinkage occurring in specimens varies on an individual basis, and wing shrinkage can vary between studies and species (Greenwood, 1979 & Harris, 1980). Based on Fjeldsa (1980) study, she found out changes of parameters occurring beyond 39 weeks before specimen preparation and related this upon drying effect.

Often, museum specimen are used to determine the sex of monomorphic birds. However due to preservation process of specimen, every body part of the specimen have the potential to decrease in size which also known as shrink. Beside, several factors such as time of preservation, species age and preservation technique may affect the shrinkage of the specimens.

Preview studies shown that there are several aspect or part of bird museum specimen that experienced obvious shrinkage. First, the primary parameter measures of the bull was exposed culmen (Parkes, 1988). Thus, drying may affect the head skin to retract, exposing more of the culmen and result in lengthening this character in museum specimen. Fjeldsa (1980) stated that shrinkage in bill length will vary with bill morphology. According to Winker (1993), specimen drying has resulted in bill length changes varying from an increase in length of 1% to a decrease of 4%. Proven shrinkage change by 1.8% difference was reported for upper

mandible by Smith *et al.* (1995).

Other than that, references offered in Greenwood (1979), who found an average wing length shrinkage in Dunlin (*Calidris alpina*) of 1%. In addition to other species, average wing shrinkage also found of 1.1% in Puffins (*Fratercula arctica*) (Harris, 1980), 1.2% in Rooks (*Corvus frigilegus*) (Knox 1980), 2% in Black Guilemots (*Cepphus grylle*) (Ewins, 1985), 2.7% and 2.2% in Ringed Plovers (Charadrius hiaticula) and Dunlin (Green, 1980). This shown that degree of obvious shrinkage will appear to old and dry specimen and vary for every species. Jenni and Winkler (1989) implies an approximately 4% wing shrinkage in many species of passerine. Another shrikage in wing length was found on Willow Tits (*Parus montanus*) for about 1.1-1.2% (Haftorn, 1982).

Tail and tarsus length measurement stated to be less prone to skrinkage than wings, because they typically does not include bone joints. Based on Dunlin species (Greenwood, 1979) and various grebe species (Fjeldsa, 1980) research, they found insignificant degree of tarsus shrinkage, but Bjordal (1983) found significant tarsus length shrinkage in House Sparrows for 1.1-1.3% and Herremans (1985) found that tarsus length increased significantly in a sample of two *Hypsipetes* species or 0.9%. Tail Shrinkage of Dunlin was found to be 2.4% (Greenwood, 1979) and he concluded it might be because of the result of pressing measuring device into live (soft) versus dead (hard) flesh.

Variable amounts of shrinkage between species and between sexes across species have been documented for museum specimen. This shrinkage has been found to be less significant in measurement such as culmen and tarsus that do not involve joints or ligaments (Winker, 1993).

Table 1 : Summary of several factors in changes in body measurement reported by several researchers

Factors	Result	References
Drying	Bill length changes varying from an increase of 1% to decrease of 4%	Parkes (1988) Winker (1993) Fjeldsa (1980) Bjordal (1983)
	Decrease in average wing length ranging from 0.5% to 4%	Greenwood (1979) Harris (1980) Knox (1980) Ewins (1985) Fjeldsa (1980) Bjordal (1983) Herremans (1985) Jenni and Winkler (1989) Hafton (1982)
	Increase in tarsus length changes ranging from 0.9% to 1.3%	Herremans (1985) Bjordal (1983) Haftorn (1982)
	Tail length changes varying from an increase of 1.5% to a decrease of 1.2%	Herremans (1985) Bjordal (1983) West *et al.* (1968) Haftorn (1982)
Assumption of pressing effect from measuring device	Shrinkage in tail length was found to be 2.4%	Greenwood (1979)
Tying of bill	Decrease in bill width length	Wilson and McCracken (2008)
Evolutionary response to dietary shift	Shortening of the upper mandible of Hawaiian honeycreeper	Smith *et al.* (2008)

2.3 Statistical Packace for Social Science (SPSS)

SPSS is a software that used for statistical analysis that allow handling large amounts of data and perform analyses covered in text and much more. It is windows based program that can be used to perform data entry and analysis and to create tables and graphs. According to Brian (2008), SPSS data file for the independent *t* test requires two variable where one variable, the grouping variable, represent the value of the independent variable while the second variable represent the dependent variable, and for addition, the grouping variable should have two distinct value (0 for a control group and 1 for experimental group).

2.3.1 Independent Sample t-test

In this study, An independent samples t-test is used for comparing the means on an interval variable between two categories on a nominal variable. In other word, the independent-sample *t* test compares the mean of two samples. Both of the sample are normally from randomly assigned groups and both groups being compared should be independent of each other. One group is independent to the other only when they are unrelated to each other. To accomplish independent group, random assignment need to be perform (Brian, 2008). Brian also stated that dependent variable must be measured on an interval or ratio scale and as for independent variable, it should have only two discrete levels.

According to de Huck (2004), small sample size do not cause a problem or fundamental objection when conducting a t-test as long as the effect size is large. For example, significant difference between two sample in length of pelvic par between male and female was analysed using independent t-test (Sachdev *et al.*, 2014).

The following equation is the formula for independent t-test:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}} \quad \text{.....(1)}$$

X_1 = Mean value of first group

X_2 = Mean value of secobnd group

S_1 = Standard deviation of first group

S_2 = Standard deviation of first group

N_1 = Sample size of first group

N_2 = Sample size of second group

2.3.2 Mann-Whitney U test

Yue and Wang (2002) stated that Mann-Whitney U test statistically used for assessing the significance of a shift in median or mean requires a tested series to be serially independent. It is also stated that Mann-Whitney U test sometimes referred to as a test of medians, or nonparametric version of the two sample t-test (Fagerland & Sandvik, 2009). In other word, Mann-Whitney is very useful to identify whether two independent set of data show significant difference or not in the selected variable. Mann-Whitney statistical analysis does not required large sample and it has great advantage of being used for small sample of group (Nachar, 2008). Is is also known that Mann-Whitney U test could not be a good analysis to be used for a large sample study to compare means or median of two population (Fagerland & Sandvik 2009). Milenkovic (2011) stated that Mann-Whitney U test is a non-parametris test used to compare between two independent groups. Mann-Whitney U test is different from

independent t-test as independent t-test is a parametric test to compare mean values between to two group while Mann-Whitney U test is a non-parametric test used to compared median between two group (Milenkovic, 2011).

The following equation is the formula for Mann-Whitney U:

$$U_a = n_a n_b + \frac{n_a(n_a+1)}{2} - \sum R_a$$

and

$$U_b = n_a n_b + \frac{n_b(n_b+1)}{2} - \sum R_b \qquad \text{.....(2)}$$

R_1 = Rank sum for the sample a

R_2 = Rank sum for the sample b

n_1 = Number of data in sample a

n_2 = Number of data in sample b

3.0 Materials and methods

In this study, two type of data were used which were primary data which involve sampling for live specimen and secondary data obtained from previous studies.

3.1 Location of Sampling Site (Primary data)

Study areas were divided into 2 sites which are site A and site B. Our sampling for live-fresh specimen was conducted at Gading National Park, Lundu, Sarawak (1°43.00' N, 110° 50.20' E) for over 5 consecutive days from 19th October 2015 until 23rd October 2015 for site A and 14th January 2015 until 19th January 2015 for site B. The study was conducted at main camp site of Gading National Park and Sungai Sepalir area which located behind the mount near oil palm plantation. This oil palm plantation is under FELCRA, Lundu.

Figure 1 : Location of Mount Gading(Sampling Site) in Kuching (source : http://maps.google.com.my/)

3.2 Field Methods

During sampling, 4 olive-winged bulbul, 4 yellow-vented bulbul and 4 puff-backed bulbul was caught. The methods used to collect the data of live specimen for this 3 species of bulbul in this National Park is mist-netting.

3.2.1 Mist-netting

Mist-nets were normally made of nylon mesh suspended between two poles, similar to an oversized volleyball net (Sodhi *et al.*, 2004). The mist nets were set up with extendable aluminium poles. In this study, the mist net was set up at two different type of forest at primary forest and secondary forest. The total numbers of mist-net used for both area were 20 until the end of sampling day. The poles were set on the ground with the lowest shelf being about one meter above the ground. The mist-nets were set from 0600 hr to 1800 hr and were checked regularly at two hours interval. To avoid risk of captured bird exposed to ground predators, the mist nets were set up at least 0.5 m above the ground (Arif & Mohd Azlan, 2014).

The captured birds were placed in cloth bag. The morphological data of the bird captured were recorded in a data sheet for further analysis (Appendix 1). Measurement of birds that were recorded include weight, tarsus (TR), bill length (BL), bill depth (BD), bill width (BW), head bill (HB), wing length (WL), wingspan (WS), tail (TA), and total length (TL). The weights of recorded birds were measured using Pesola spring balance (10g, 50g, 100g, and 300g). At the same time, the measurement of birds were measured using digital caliper and ruler. Identification of the birds was done using MacKinnon and Phillips (1993) and Smythies (1999). Before the birds were released, the birds were ringed on their right legs.

3.3 Data Collection for Live Specimen as Secondary Data

Secondary data for live specimen were taken from previously published and unpublished report. Table below are the summary of secondary data collected:

Table 2: Summary of Data Collected from Previous Studies

Type of Data	Researchers	Research Title	Data Collected
Secondary Data	Nur Azirah Binti Mohammad Arif (2012)	The Diversity of Understorey Birds at Gunung Gading National Park, Sarawak	3 olive-winged bulbul 2 yellow-vented bulbul
	Nor Azlini Bt Mat Isahak Zaki (2013)	The Diversity of Understorey Birds in Oil Palm Plantation, Kota Samarahan, Sarawak	2 olive-winged bulbul 2 yellow-vented bulbul
	Nurhafizie Binti Mohamad Hapiszudin (2008)	The Diversity and Abundance of Understorey Avifauna at Universiti Malaysia Sarawak (UNIMAS) Campus, Kota Samarahan, Sarawak	13 olive-winged bubul 2 puff-backed bulbul
	Nurul Syuhada Binti Abdillah (2011)	Diversity of Avifauna in Universiti Malaysia Sarawak (UNIMAS) Campus, Kota Samarahan	9 olive-winged bulbul
		Total	27 olive-winged bulbul 4 yellow-vented bulbul 2 puff-backed bulbul

3.4 Data Collection for Museum Specimen

Museum specimen data were obtained from previous study conducted by Siti Fairuz (2007) with the title of "Logistic Regression Analysis To Determine The Gender Of Bulbul (Family : Pycnonotidae) Using External Morphological Characters. From her study, I managed to obtained 54 olive-winged bulbul (*P. plumosus*), 29 puff-backed bulbul (*P. eutilotus*) and 75 yellow-vented bulbul (*P. goiavier gourdini*). Due to lack of data for tail and weight parameter for bulbul museum specimen, both parameter were not included for data analysis.

3.5 Measurement

The measurement of morphological features were conducted using bulbul's live and museum specimen. Digital calipers and stainless steel ruler were used to measure the specimen and the data was recorded in the data sheet. The seven morphological features that had been measured are shown in Table 3.

Table 3: List of measurements (mm).

	External Features	Description
1	Tarsus (TR)	From below the thigh until the palm.
2	Bill length (BL)	From the middle of the nostril till the edge of the bill.
3	Bill width (BW)	Across the bill from left to right of the nostrils.
4	Bill depth (BD)	Across the bill vertically at the nostrils.
5	Head to bill length (HB)	From the back of the head until the edge of the bill.
6	Wing length (WL)	From edge of wing until the edge of longest feathers.
7	Total length (TL)	From the edge of the bill until the edge of the longest feather on the tail, with the bird lying on its back.

Figure 2 shows the measurement of external morphological characters for birds. The juveniles were excluded from these measurement to avoid bias (Reduan *et al.*, 2003). According to Pyle *et al.* (1993), the passerines juvenile primaries tend to be slightly (2-5%) shorter than adult primaries. So for that this study the juveniles were excluded.

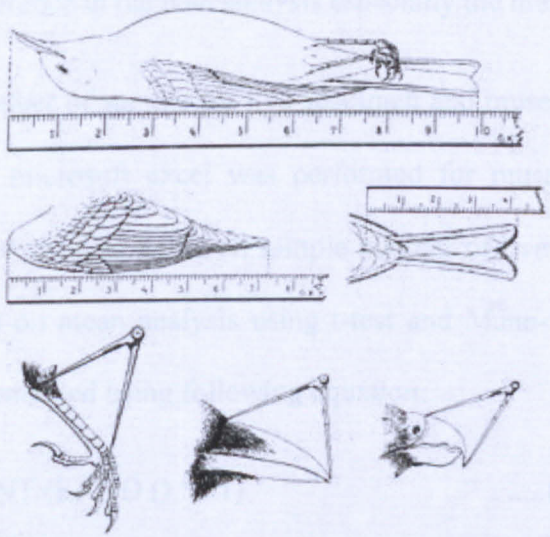

Figure 2 : The external morphology characters measurement (McClure, 1988)

Description of Parameters

Top : Total Length (TL)

Left : Tail (TA) / Right : Wing Length (WL)

Center and Left : Bill Length (BL) / Right : Tarsus (TR)

3.6 Data Analysis

The number of sample for museum specimen was not the same as number of sample of live specimen from primary data and secondary data which is total of 31 olive-winged bulbul, 8 yellow-vented bulbul and 6 puff-backed bulbul. Different number of sample between two group will cause big difference in the data analysis especially the mean of two group.

Due to differences in number of sample for live specimen and museum specimen, the random number generator using microsoft excel was performed for museum specimen species to choose random number of samples based on sample number of live specimen caught. This is to prevent error and bias on mean analysis using t-test and Mann-Whitney U test. Random Number Generator was generated using following equation:

$$= \text{INT (RAND ()} * 31) \qquad \text{.....(3)}$$

Thirty-one samples were randomly selected for olive-winged bulbul while eight samples for puff-backed bulbul and six samples for yellow-vented bulbul from the museum data by Fairuz (2007) to exact the number of sample from live-traping, Independent sample t-test was used for olive-winged bulbul to measure the different in mean value between two independent groups.

The data for comparison of museum and live specimen was analyzed using Statistical Package Social Sciences (SPSS). All parameters of live specimen and museum specimen except weight and tail were analyzed with independent-t test. Each parameter of museum and live specimen were compared to find their significant differences.

While for puff-backed bulbul only with 6 sample and yellow-vented bulbul only with 8 sample were available for museum and fresh samples. Since the numbers of samples were limited, the significant difference between live-fresh and museum specimen were evaluated using Mann-Whitney U test.

The percentage of shrinkage in each body measurement for all three species were assessed using the mean difference of live-fresh and museum specimen. The equation to find the percentage of shrinkage is:

$$\frac{\text{Mean length of live specimens - mean length of museum specimens}}{\text{Mean length of live specimens}} \times 100 \quad \text{.....(6)}$$

4.0 Results

The seven parameters that had been measured for the Sarawak Museum specimen and live specimen included bill length (BL), bill width (BW), bill depth (BD), head to bill (HB), wing length (WL), tarsus (TR), total length (TL). There are three species of bulbul included which is *Pycnonotus pulmosus* (olive-winged bulbul), *Pycnonotus goivier gourdini* (yellow-vented bulbul) and *Pycnonotus eutilotus* (puff-backed bulbul). All measurement of morphological characteristic for the three species showed various percentage of shrinkage. The significant difference of measurement was assess using P-value range of 2-tailed from 0.05 to -0.05. Any 2-tailed t-test within the range will be considered to have a significant.

Table 4 : Number of live and museum specimen for each species of bulbul after Random Number Generator Step

Species	Number of individual for museum specimen	Number of individual for live specimen	Total
Pycnonotus pulmosus	31	31	62
Pycnonotus goivier gourdini	8	8	16
Pycnonotus eutilotus	6	6	12
	45	45	90

4.1 Olive-winged Bulbul (*P. plumosus*)

Table 5 : Summary on descriptive statistics and percentage of shrinkage of olive-winged bulbul (*P.plumosus*)

Parameter	n	Mean length (mm)		t^a	p value(2-tailed)	Mean Difference	Std. Error Difference	% Shrinkage
		Live	Museum					
Tarsus	31	22.22	21.63	1.25	0.460	0.59	0.48	2.66
Bill-length	31	12.00	11.55	0.94	0.353	0.45	0.49	3.75
Bill-width	**31**	**4.85**	**4.20**	**2.95**	**0.005**	**0.65**	**0.22**	**13.40**
Bill-depth	31	5.23	4.88	1.84	0.071	0.35	0.19	6.69
Head-bill	**31**	**38.78**	**35.32**	**4.18**	**0.000**	**3.46**	**0.83**	**8.92**
Wing-length	31	83.32	79.27	2.28	0.216	4.05	1.78	4.86
Total-length	31	166.94	166.35	-0.15	0.886	0.59	4.02	0.35

Based on the table above, all the seven parameters of the olive-winged bulbul show decrease in length (mm). Bill-width showed highest percentage of shrinkage (13.40%) followed by head-bill (8.92%), bill-depth (6.69%), wing-length (4.86%), bill-length (3.75%) , tarsus (2.66%) and the lowest was total-length (0.35%).

There are two body parts of olive-winged bulbul (*P.plumosus*) that showed a significant different between museum and live-fresh specimen which is bill-width ($t^a = 2.95$, $p < 0.05$), and head-bill ($t^a = 4.18$, $p < 0.05$). While bill-length ($t^a = 0.94$, $p > 0.05$), bill-depth ($t^a = 1.84$, $p > 0.05$), wing-length ($t^a = 2.28$, $p > 0.05$), tarsus ($t^a = 1.25$, $p < 0.05$) and total-length ($t^a = -0.15$, $p > 0.05$) does not show a significant difference in term of measurement.

4.2 Puff-backed Bulbul (*P.eutilotus*)

Table 6 : Summary on descriptive statistics and percentage of shrinkage of puff-backed bulbul (*P.eutilotus*)

Parameter	n	Mean Length (mm)		Mann-Whitney U	p value(2-tailed)	Mean Difference	% Shrinkage
		Live	Museum				
Tarsus	6	20.74	20.24	15.000	0.631	0.50	2.41
Bill-length	**6**	**11.61**	**13.55**	**0.000**	**0.004**	**-1.94**	**-16.71**
Bill-width	6	5.59	5.08	8.000	0.109	0.51	9.12
Bill-depth	6	5.72	5.53	11.500	0.297	0.19	3.32
Head-bill	6	38.04	37.14	14.000	0.521	0.9	2.37
Wing-length	6	94.33	91.90	8.000	0.109	2.43	2.58
Total-length	6	193.67	186.67	13.000	0.422	7.00	3.61

Mean of all the 7 body part for puff-backed bulbul (*P.eutilotus*) showed different value of shrinkage. Bill-width showed the most highest percentage of shrinkage (9.12%), followed by total-length (3.61%), bill-depth (3.32%), wing-length (2.58%), head-bill (2.37%) and lastly tarsus (2.41%).

Based on the critical value table for Mann-Whitney U test, the critical value for this analysis was 5 when the sample size were 6 for both groups. Out of seven body measurement, there is only one parameter that was significant difference. The parameter are bill-length ($U = 0.000$, $p < 0.05$). Other body measurement include tarsus ($U = 15.000$, $p > 0.05$), bill-width ($U = 8.000$, $p > 0.05$), bill-depth ($U = 11.500$, $p > 0.05$), head-bill ($U = 14.000$, $p > 0.05$), wing-length ($U = 8.000$, $p > 0.05$), and total-length ($U = 13.000$, $p > 0.05$) does not showed any significant different between museum and live-fresh specimen.

4.3 Yellow-vented Bulbul (*P. goiavier gourdini*)

Table 7 : Summary on descriptive statistics and percentage of shrinkage of yellow-vented bulbul (*P. goiavier gourdini*)

Parameter	n	Mean Length (mm)		Mann-Whitney U	p value(2-tailed)	Mean Difference	% Shrinkage
		Live	Museum				
Tarsus	8	20.44	20.13	25.000	0.462	0.31	1.52
Bill-length	**8**	**14.42**	**11.10**	**7.000**	**0.009**	**3.32**	**23.02**
Bill-width	**8**	**4.13**	**3.66**	**9.000**	**0.016**	**0.47**	**11.38**
Bill-depth	**8**	**5.65**	**4.10**	**0.000**	**0.001**	**1.55**	**27.43**
Head-bill	**8**	**37.79**	**33.41**	**9.000**	**0.016**	**4.38**	**10.53**
Wing-length	8	77.25	75.88	21.000	0.248	1.37	1.77
Total-length	8	188.50	167.63	15.500	0.082	20.87	11.07

All of the measured component showed significant change. All component of yellow-vented bulbul (*P. goiavier gourdini*) showed a decrease in length. A highest percentage of shrinkage can be seen in bill-depth with 27.43% and the lowest is in tarsus which is 1.52%. Bill-width and head-bill showed a decrease in length same as for olive-winged bulbul (*P.plumosus*) species involve in this study.

Based on the critical value table for Mann-Whitney U test, the critical value for this analysis was 13 when the sample size were 8 for both group. Referring from above table, bill-length ($U = 7.000$, $p < 0.05$), bill-width ($U = 9.000$, $p < 0.05$), bill-depth ($U = 0.000$, $p < 0.05$) and head-bill ($U = 9.000$, $p < 0.05$) was less than 13 indicated that there was significant difference in this 4 parameters. Bill-depth ($U = 0.000$, $p < 0.05$) showed highest significant differences with the value of 0.001. However, tarsus, wing-length and total-length do not show any significant difference with $p > 0.05$.

5.0 Discussion

Determination of sex of birds of monochromatic or dimorphism species are frequently developed using museum specimen based on their parameter measurement. Shrinkage tend to occur as a natural process within all museum specimen where researcher typically ignored it and result of museum based study are applied directly to live-fresh specimen. Based on previous study by Fjeldsa (1980), shrinkage can reach an average of 4% which seems unwise to ignore. The shrinkage of the museum specimen can be determined by comparing it with live-fresh specimen using statistical analysis.

Live specimen for olive-winged bulbul species reach the above 30 individuals which is about enough to the analysis using t-test, while the live specimen for two species; puff-backed bulbul and yellow-vented bulbul could not be use for t-test but instead it is analyzed using Mann-Whitney U test because of the sensitivity of the Mann-Whitney U test to analyzed group of small sample below 30 individuals. Olive-winged bulbul has 31 individuals while 8 individuals for yellow-vented bulbul and 6 individuals for puff-backed bulbul.

According to Reduan *et al.* (2003), eight parameters have frequently been used for study of bulbuls especially the study to differentiate the gender of bulbul. As for the measurement of secondary data for museum specimen. only seven parameters were included where the measurement of tail length (TA) was eliminated from the list of parameters because since the specimen at the Sarawak Museum were old, it cannot be forced while doing the measurement which might damage the specimens (Fairuz, 2007). Besides that, wing span also excluded since there is no standard measurement for the dry specimen.

In this study, comparison of body measurement for museum and live-fresh specimen was done to obtain the percentage of shrinkage and its significant different where it is important as certain or most museum specimen may shrink and researchers prone to exclude the shrinkage value. Shrinkage usually start directly following death (Morison, 2004) and most common factors of shrinkage is due to water loss (Wessels, 2009). There was also other factors which lead to changes in length of body part such as pressing effect from measuring device (Greenwood, 1979), retraction of skin stated by Bjordal (1983) and Herremans (1985) and preparation step such as tying of bill stated by Wilson and McCracken (2008). Based on the current literature, there is no previous study that can proved that aging of specimen will cause more shrinkage. Fjeldsa (1980) found no changes in length beyond 39 weeks after specimen preparation.

Measurements of tail, bill and tarsus lengths might be less prone to shrinkage than wings, because they do not (typically) include bone joints. In this study olive-winged bulbul (*P.plumosus*), showed significant difference in length of its external morphology which include tarsus, bill-width and head-bill. As for puff-backed bulbul (*P.eutilotus*), only bill-length showed significant difference while yellow-vented bulbul (*P. goiavier gourdini*), showed significant difference in bill-length, bill-width, bill-depth and head-bill.

Besides drying, preparation of specimen may also contribute to the differences between measurement. In this study, **bill-width** of olive-winged bulbul (*P.plumosus*) showed significant different in length with $p(0.005) < 0.05$ and in addition it showed the highest percentage of shrinkage with 13.40% compared to other body measurement. Same as

olive-winged bulbul, yellow-vented bulbul(*P. goiavier gourdini*) also showed significant different for bill-width and the percentage tend to be quite high with 11.38%. Previous study made by Wilson and McCracken (2008) stated that Cinnamon Teal also have significant difference with $p(0.001) < 0.05$ and also have the highest percentage shrinkage among all other component. Based on Wilson and McCracken (2008), bills of specimen were tied to keep them close during the process of drying, this may have squeezed the bill together resulting in decreasing of bill-width. Thus, this could be the reason why bill-width prone to shrink a lot and have significant difference.

One of important morphology for studying feeding ecology and subspecies classification is **bill length** (Hall, 1996). Thus, it is vital to have an accurate bill measurement. Therefore to ignore shrinkage for length of bill is unwise. Shrinkage in bill length varies with bill morphology (Fjeldsa 1980). Result in this study showed puff-backed bulbul (*P.eutilotus*) with ($U = 0.000$, $p < 0.05$) which have significant different but the mean length experiencing increase in length measurement rather than decreasing. Previous study by Winker (1993) also showed an increase in the bill length of Tennessee Warbler. The increase might be due to shrinking and hardening of the skin that forms the anterior edge of the nasal fossae (Winker, 1993). This prevent calipers from measuring more anteriorly within the nares. Other than that, Winker also mentioned the reason from increasing might due to drying effect that cause the head skin to retract posteriorly. As for yellow-vented bulbul (*P. goiavier gourdini*), bill-length have statistic of ($U = 7.000$, $p < 0.05$) and shrinkage of 23.02%. The shrinkage is utterly big, in addition of it being significant different. Fjeldsa (1980) found shrinkage of 4% in grebe bills. There are many ways to measure bill length which are total culmen length, exposed culmen

length and length from the nares (Baldwin *et al.*, 1931). Fjeldsa (1980) suggested that amoung of shrinkage will be different according to bill structure and this has led to the recommendation of measuring bill length from the posterior edge of the nares as it is more easily defined (Winker 1998, Borras *et al.*, 2000). Study by Wilson and McCracken (2008) confirms the recommendation in waterfowl, that measurement of bill length from nares is more reliable.

Head bill also found to shrink and have significant different for both olive-winged bulbul and yellow-vented bulbul. Head bill standard measurement involve the bill length itself. Therefore, the shrinkage occur on the bill length might affect the head bill length which found on yellow-vented bulbul result. Other than that, the changes may also occur due to non-standardized measuring technique.

Some body measurement that were found to have significant change in previous study and were not found in this study were wing length, tarsus length and lastly tail length which not include in this study due to lack of tail measurement from secondary museum data. Greenwood (1979) found insignificant change in **tarsus length** of Dunlin species, but Bjordal (1983) found significant tarsus length shrinkage in House Sparrows (1.1 - 1.3%) while Herremans (1985) found tarsus length of two sample *Hypsipetes* to increased significantly (0.9%). Bjordal (1983) stated that the shrinkage might be because of the thinning of the tissue at the proximal end of the tarsus during drying. **Tail length** shrinkage in Dunlin found by Greenwood (1979) to be 2.4% and suggesting it might due to pressing force of measuring device into live-soft and dead-hard flesh. However, Bjordal (1983) and Herremans (1985)

found increased in tail length after drying process in House Sparrows (0.4 - 1.5%) and *Hypsipetes* species (1.1%). Both authors thought this result from retraction of intercalamal skin during drying. Winker (1993), suggested that the differences in the tail measurement of all three species might be because of different preservation technique.

As for **wing length**, it supposed to be the body part that have a most potential to shrink because one of the factors is, it consists of bone joint. Study done by Greenwood (1979), Harris (1980), Knox (1980), Ewins (1985), Fjeldsa (1980), Bjrodal (1983) and Herremans (1985) who found the shrinkage in wing length of different species which range from 0.5 to 4% respectively. It is stated that primary feather lengths were immune to shrinkage and would be superior for wing length measurement (Jenni and Winkler 1989), however chord might be more reliable for some species (Winker, 2013) although wing chord was found to have significant difference in p value [$p(0.001) < 0.05$] with 2.12% of shrinkage (McCracken 2008).

Besides all the reason regarding shrinkage, there is one more reason that have been reported by Smith *et al.* (1995), the historic shortening of the upper mandible of an Hawaiian honeycreeper which is viewed changes as an **evolutionary response** to a dietary shift necessitated by the widespread declines and extinctions of lobeloids (food source). However, Winker (1993), viewed their result as an artifact of specimen shrinkage, rather than evolutionary changes.

Based on the result of this study, amount of shrinkage for each body measurement were found ranging from 0.35% to 13.40% for olive-winged bulbul and -16.71% to 9.12% for puff-backed bulbul while for yellow-vented bulbul ranging from 1.52% to 27.43%. The percentage were quite high compared to Winker (1993) and Wilson and McCracken (2008). In Wilson and McCracken (2008), the shrinkage were found ranging from 6.44% to -0.03% while in Winker (1993) the shrinkage range around 3.73% to -0.96%.

Nevertheless, before applying museum-derived sexing guidelines to living specimen, researchers should correct their data for specimen shrinkage. To do this. Measurement of live specimen should be multiplied by some value suggested by Greenwood (1979) which is 0.960 to 0.996 depending on species and their anatomy. A general correction for wing where shrinkage yet to be investigated has been suggested by Greenwood (1979) which approximately 0.983. More study and data of other bird could be useful.

6.0 Conclusion and Recommendation

Amount of shrinkage and it significant difference was found various among species and their morphology. The significant difference were found in bill width and head bill of olive-winged bulbul (*P.plumosus*), bill length of puff-backed bulbul (*P.eutilotus*) and bill length, bill width, bill depth and head bill of yellow-vented bulbul(*P. goiavier gourdini*). As for the amount of shrinkage, it were found ranging from 0.35% to 13.40% for olive-winged bulbul, -16.71% to 9.12% for puff-backed bulbul and 1.52% to 27.43% for yellow-vented bulbul.

It is recommended that more study on this species is be done to analyze the universal correction factor of the external morphological characteristic for all species. The **number of individuals should be added more** in order to gather more accurate result. Further study using live specimen to measure and keep it in a museum for certain period of time to measure it again should be done to know the exact percentage of shrinkage. Through that, aging factors could also be determine.

Other than that, is it recommended that **study on other species of bulbul** is made to test whether same species have different amount or equaled amount of shrinkage and significant difference. It is stated that, different species will have different amount of shrinkage based on the anatomy of the body.

For more accurate information regarding shrinkage, **long-term shrinkage study for this three species should be done**. For example, live specimen of the three species involving 30

individual will be caught and measured based on the 7 parameters, and skinned to be kept dry in normal museum condition. Every 2 weeks, the three species will be measured to check if there is any decrease in the length of all the 7 parameters. This is done until the decrease is halted which reported will be around 39 weeks after the process of specimen preparation in the museum. Through this, accurate percentage of shrinkage could be determine.

It is also recommended to **determine specific correction factors** for this three bulbul species. This can be done with more information and accurate data measurement from large sample. Using standardized measuring technique for both live and museum specimen could reduce the error of measurement for all the parameter cause by different measurer. From the difference of mean between both sample, specific correction factors could be apply to museum to enable any measurement study of museum specimen using this bulbul species be applied directly to live specimen.

For more advance study, It is recommended to use a **more reliable method of measurement** for live-fresh and museum specimen. For example, measurement of wing length should be measured based on the primary feathers because Jenni and Winkler (1989) found that primary feather lengths were immune to shrinkage and would be superior for wing length measurement. Other than that, recommendation on measurement of bill-length from the nares to the tip of the bill because exposed culmen tend to shrink overtime. Wilson and McCracken (2008) confirms the that measurement of bill length from nares is more reliable

7.0 References

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Appendices

Appendix 1

Live Specimen Data of Olive- Winged Bulbul (*Pycnonotus plumosus*)

DATE	LOCALITY	RING NO	COMMON NAME	WT	TR	BL	BD	BW	HB	WL	WS	TA	TL
20-10-14	Gading NP	A11288	Olive- winged Bulbul	14.0	19.32	13.10	4.15	4.63	56.70	67.0	215.0	44.0	158.0
20-10-14	Gading NP	B3320	Olive- winged Bulbul	33.0	21.09	11.43	5.29	5.42	38.14	81.0	230.0	79.0	170.0
20-10-14	Gading NP	A10173	Olive- winged Bulbul	11.5	16.5	17.7	3.62	4.20	41.12	65.0	165.0	47.0	133.0
23-10-14	Gading NP	B3326	Olive- winged Bulbul	32.0	23.40	10.97	3.00	2.44	37.52	85.0	240.0	77.0	166.0
28-01-12	Gading NP	A10165	Olive- winged Bulbul	24.0	19.9	10.13	5.0	3.0	35.0	80.0	240.0	51.0	169.0
28-01-12	Gading NP	A10170	Olive- winged Bulbul	29.0	19.42	13.30	5.27	4.0	37.9	84.0	164.0	74.0	169.0
10-11-11	Gading NP	C05005	Olive- winged Bulbul	45.0	22.15	14.76	8.0	7.5	49.0	107.0	330.0	91.0	182.0
31-07-10	UNIMAS	-	Olive- winged Bulbul	25.0	21.84	12.68	5.22	4.85	38.9	81.0	225.0	0	121.0
31-07-10	UNIMAS	-	Olive- winged Bulbul	28.0	17.85	9.43	3.36	4.98	35.0	78.0	210.0	77.0	180.0
31-07-10	UNIMAS	-	Olive- winged Bulbul	28.0	20.72	11.74	4.87	4.29	39.0	78.0	246.0	74.0	160.0
03-08-10	UNIMAS	B3750	Olive- winged Bulbul	29.0	17.65	15.31	5.42	5.74	39.0	90.0	160.0	150.0	145.0
04-08-10	UNIMAS	B1019	Olive- winged Bulbul	32.0	22.51	11.83	6.27	4.76	40.0	87.0	262.0	81.0	186.0
04-08-10	UNIMAS	B1020	Olive- winged Bulbul	28.0	24.32	9.6	5.38	5.62	37.0	80.0	248.0	78.0	160.0
04-08-10	UNIMAS	-	Olive- winged Bulbul	30.0	22.77	12.44	5.59	5.98	40.0	78.0	27.1	77.0	161.0

04-08-10	UNIMAS	B1021	Olive- winged Bulbul	25.0	23.53	11.58	4.68	4.62	40.0	76.0	238.0	82.0	158.0
30-11-10	UNIMAS	-	Olive- winged Bulbul	27.0	19.70	11.22	5.79	4.73	37.22	80.0	221.0	69.0	178.0
28-07-07	UNIMAS	B3598	Olive- winged Bulbul	26.0	23.0	11.0	5.25	4.36	37.73	80.0	230.0	60.0	165.0
28-07-07	UNIMAS	B3599	Olive- winged Bulbul	29.1	22.2	12.1	5.03	4.52	37.9	88.0	252.0	75.0	175.0
28-07-07	UNIMAS	B3597	Olive- winged Bulbul	29.1	23.1	10.7	5.06	4.24	38.14	79.0	245.0	75.0	175.0
28-07-07	UNIMAS	B3596	Olive- winged Bulbul	31.0	25.2	12.0	5.09	3.25	34.55	83.0	260.0	86.0	183.0
28-07-07	UNIMAS	A3726	Olive- winged Bulbul	31.0	24.2	10.6	5.6	5.61	37.84	87.0	210.0	77.0	180.0
28-07-07	UNIMAS	B3595	Olive- winged Bulbul	28.0	24.0	12.3	4.8	4.05	35.63	85.0	245.0	76.0	183.0
28-07-07	UNIMAS	B3594	Olive- winged Bulbul	30.0	22.79	11.0	4.13	3.34	35.05	80.0	240.0	75.0	170.0
28-07-07	UNIMAS	A3728	Olive- winged Bulbul	29.0	24.1	10.5	5.42	5.42	37.28	82.0	258.0	83.0	181.0
29-07-07	UNIMAS	A3739	Olive- winged Bulbul	32.0	20.6	13.2	5.97	5.97	37.25	85.0	269.0	84.0	183.0
29-07-07	UNIMAS	A3740	Olive- winged Bulbul	33.0	23.6	13.9	5.68	6.68	38.7	84.0	160.0	83.0	178.0
29-07-07	UNIMAS	A3741	Olive- winged Bulbul	31.0	21.0	11.1	6.05	6.03	35.17	84.0	261.0	76.0	176.0
18-08-07	UNIMAS	A2397	Olive- winged Bulbul	34.0	22.6	13.2	5.88	5.57	40.64	115.0	260.0	84.0	170.0
18-08-07	UNIMAS	A2353	Olive- winged Bulbul	27.0	17.9	10.1	5.35	2.72	37.23	84.0	266.0	78.0	178.0
28-11-07	UNIMAS	B1078	Olive- winged Bulbul	31.5	18.8	11.5	5.87	5.96	39.58	88.0	560.0	78.0	171.0
23-11-07	UNIMAS	B1077	Olive- winged Bulbul	32.0	24.7	11.6	5.9	5.99	38.11	82.0	248.0	73.0	93.0

Appendix 2

Live Specimen Data of Puff-backed Bulbul (*Pycnonotus eutilotus*)

DATE	LOCALITY	RING NO	COMMON NAME	WT	TR	BL	BD	BW	HB	WL	WS	TA	TL
28-07-07	UNIMAS	B3567	Puff-backed bulbul	32.0	23.7	12.8	5.50	5.9	39.27	96.0	274.0	103.0	204.0
27-10-07	UNIMAS	B3445	Puff-backed bulbul	38.0	15.8	9.85	5.95	5.58	37.17	92.0	256.0	74.0	188.0
28-10-07	UNIMAS	B3132	Puff-backed bulbul	34.0	21.0	10.70	5.87	5.97	37.0	93.0	262.0	78.0	189.0
23-11-07	UNIMAS	B3134	Puff-backed bulbul	35.0	22.0	11.70	5.34	5.96	38.3	94.0	264.0	76.0	190.0
24-11-07	UNIMAS	B3138	Puff-backed bulbul	37.50	21.95	12.46	5.88	5.08	39.27	95.0	265.0	74.0	194.0
25-11-07	UNIMAS	B3333	Puff-backed bulbul	38.0	16.99	12.17	5.79	5.02	37.25	96.0	272.0	81.0	197.0

Appendix 3

Live Specimen Data of Yellow- vented Bulbul (*Pycnonotus goivier gourdini*)

DATE	LOCALITY	RING NO	COMMON NAME	WT	TR	BL	BD	BW	HB	WL	WS	TA	TL
31-01-12	Gading NP	A10166	Yellow- vented bulbul	36.0	21.12	17.29	6.93	4.71	44.0	61.0	293.0	87.0	221.0
31-01-12	Gading NP	A10167	Yellow- vented bulbul	37.0	21.10	17.19	7.03	4.55	43.0	62.0	293.0	86.0	222.0
24-04-13	FELCRA, Samarahan	A2565	Yellow- vented bulbul	24.0	21.0	10.70	5.17	3.87	33.0	78.0	262.0	78.0	189.0
28-04-13	FELCRA, Samarahan	C2259	Yellow- vented bulbul	25.0	22.0	11.70	5.70	3.86	33.3	76.0	244.0	76.0	187.0
18-01-15	SG Sepalir	B3331	Yellow- vented bulbul	29.50	21.95	12.46	5.10	4.08	38.27	88.0	295.0	174.0	174.0
19-01-15	SG Sepalir	B3333	Yellow- vented bulbul	28.0	16.99	15.17	5.17	4.02	37.25	83.0	222.0	171.0	171.0
19-01-15	SG Sepalir	B3334	Yellow- vented bulbul	28.50	18.60	16.15	5.10	4.05	37.80	88.0	295.0	173.0	173.0
19-01-15	SG Sepalir	B3335	Yellow- vented bulbul	27.50	18.30	14.70	5.00	3.93	35.70	82.0	220.0	171.0	171.0

Appendix 4

Critical Valus of the Mann-Whitney U
(Two-tailed test)

n_2	α	n_1																	
		3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
3	.05	--	0	0	1	1	2	2	3	3	4	4	5	5	6	6	7	7	8
	.01	--	0	0	0	0	0	0	0	0	1	1	1	2	2	2	2	3	3
4	.05	--	0	1	2	3	4	4	5	6	7	8	9	10	11	11	12	13	14
	.01	--	--	0	0	0	1	1	2	2	3	3	4	5	5	6	6	7	8
5	.05	0	1	2	3	5	6	7	8	9	11	12	13	14	15	17	18	19	20
	.01	--	--	0	1	1	2	3	4	5	6	7	7	8	9	10	11	12	13
6	.05	1	2	3	5	6	8	10	11	13	14	16	17	19	21	22	24	25	27
	.01	--	0	1	2	3	4	5	6	7	9	10	11	12	13	15	16	17	18
7	.05	1	3	5	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34
	.01	--	0	1	3	4	6	7	9	10	12	13	15	16	18	19	21	22	24
8	.05	2	4	6	8	10	13	15	17	19	22	24	26	29	31	34	36	38	41
	.01	--	1	2	4	6	7	9	11	13	15	17	18	20	22	24	26	28	30
9	.05	2	4	7	10	12	15	17	20	23	26	28	31	34	37	39	42	45	48
	.01	0	1	3	5	7	9	11	13	16	18	20	22	24	27	29	31	33	36
10	.05	3	5	8	11	14	17	20	23	26	29	33	36	39	42	45	48	52	55
	.01	0	2	4	6	9	11	13	16	18	21	24	26	29	31	34	37	39	42
11	.05	3	6	9	13	16	19	23	26	30	33	37	40	44	47	51	55	58	62
	.01	0	2	5	7	10	13	16	18	21	24	27	30	33	36	39	42	45	48
12	.05	4	7	11	14	18	22	26	29	33	37	41	45	49	53	57	61	65	69
	.01	1	3	6	9	12	15	18	21	24	27	31	34	37	41	44	47	51	54
13	.05	4	8	12	16	20	24	28	33	37	41	45	50	54	59	63	67	72	76
	.01	1	3	7	10	13	17	20	24	27	31	34	38	42	45	49	53	56	60
14	.05	5	9	13	17	22	26	31	36	40	45	50	55	59	64	67	74	78	83
	.01	1	4	7	11	15	18	22	26	30	34	38	42	46	50	54	58	63	67
15	.05	5	10	14	19	24	29	34	39	44	49	54	59	64	70	75	80	85	90
	.01	2	5	8	12	16	20	24	29	33	37	42	46	51	55	60	64	69	73
16	.05	6	11	15	21	26	31	37	42	47	53	59	64	70	75	81	86	92	98
	.01	2	5	9	13	18	22	27	31	36	41	45	50	55	60	65	70	74	79
17	.05	6	11	17	22	28	34	39	45	51	57	63	67	75	81	87	93	99	105
	.01	2	6	10	15	19	24	29	34	39	44	49	54	60	65	70	75	81	86
18	.05	7	12	18	24	30	36	42	48	55	61	67	74	80	86	93	99	106	112
	.01	2	6	11	16	21	26	31	37	42	47	53	58	64	70	75	81	87	92
19	.05	7	13	19	25	32	38	45	52	58	65	72	78	85	92	99	106	113	119
	.01	3	7	12	17	22	28	33	39	45	51	56	63	69	74	81	87	93	99
20	.05	8	14	20	27	34	41	48	55	62	69	76	83	90	98	105	112	119	127
	.01	3	8	13	18	24	30	36	42	48	54	60	67	73	79	86	92	99	105

Appendix 5

Formula for Mann-whitney U

$$U_a = n_a n_b + \frac{n_a(n_a+1)}{2} - \sum R_a$$

and

$$U_b = n_a n_b + \frac{n_b(n_b+1)}{2} - \sum R_b$$

Formula for Independent t-test

Independent t-test (formula)

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

X_1 = Mean value of first group

X_2 = Mean value of second group

S_1 = standard deviation of first group

S_2 = standard deviation of first group

n_1 = sample size of first group

n_2 = sample size of second group